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13. SUPPLEMENTARY NOTES

14. ABSTRACT Purpose/scope: Our aim is to understand whether adjuvant therapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer. Major findings: Our findings support a selection process where adjuvant systemic treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically. Up-to-date report: This is the final report

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No subject terms provided.

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1. Introduction

Globally, increasing breast cancer incidence rates, improved prognosis and growing life expectancy have resulted in increasing number of women at risk of developing a bilateral primary breast cancer. There are an estimated 2.2 million women living in the US who have been diagnosed at some time with breast cancer [1]. Hence, optimal surveillance and clinical management of women who have had one or two primary breast cancers is a challenge. However, there are only limited data on incidence rates of synchronous and metachronous breast cancer [2,3], results on temporal trends in incidence are conflicting [4] and little is known about the prognostic outlook following treatment of a second primary cancer [5,6]. Our aim is to understand whether adjuvant therapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer.

2. Body

Research accomplishments as outlined in the Statement of Work.
Task 1. Identification of cases and controls of uni- and bilateral breast cancer for the case-control data file. Month 1:

a. Using the regional cancer register in Stockholm, Sweden we will identify women diagnosed with unilateral breast cancer included in randomized clinical trails 1985-1995, n=3,200
b. Within the cohort of 3,200 unilateral breast cancer cases we will identify those diagnosed with bilateral breast cancer within 5 years of primary cancer, n=150.
c. Using the regional cancer register in Stockholm, Sweden to sample women with bilateral breast cancer within 5 years who died and those who did not, n=50+50.
 d. Identifying hospital at which medical records and tissue are located using a unique national registration number.

Summary:
Using the regional cancer register in Stockholm, Sweden we identified 17,089 women diagnosed with unilateral breast cancer, among these women, 441 developed metachronous bilateral disease within 5 years of their primary breast cancer during follow-up through 1999. We identified hospitals at which medical records and tissue are located using a unique national registration number.

Task 2. Abstracting information medical records. Months 2-4:

a. Constructing the abstracting manual.
b. Structural work of organizing a database to store information in using Oracle®
c. Retrieving medical records from hospital.
d. Abstracting information from medical records into database.
e. A database quality control program will instituted to check for errors and inconsistencies in the database.

Summary:
We constructed the abstracting manual, see Appendix 1, and completed a structural work of organizing a database to store information in using Oracle®. Of the 441 study participants with short latency contralateral breast cancer, 11% was shown at investigating of the medical record to not fulfill the inclusion criteria and was thus excluded, increasing the precision of the study. Of

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the remaining 392 patients, 97% were collected and computerized and 3% were not possible to find in the archives. We expect to finish the data collecting step during November 2008.

Regarding the tissue collection, we gained permission from the ethical committee to collect tissue from the biobank and from the biobank committee to have access to the tissue. The cases for tissue analysis were selected and ordered from the biobank, and we expected the tissue to arrive during the autumn of 2007. A pathologist and a lab technician was affiliated/involved in the project to prepare and investigate the tissue.

A database quality control program was developed to check for errors and inconsistencies in the database and for comparison of the information in the medical records and regional cancer register in Stockholm. When comparing information on treatment from medical records and from register we noticed that treatment information is (very) much more complete in the medical record than in the register. Only 2% had any of the treatment variables unknown in the dataset with information collected from medical records. Also variables regarding tumour characteristics, like tumour size, and hormonal information, like menopause status, were more complete in the information from medical records.

Task 3. Data analysis of women with bilateral breast cancer. Months 5:

a. The data analysis will be detailed and analysis of the final data set of bilateral breast cancer will be conducted using SAS statistical software package.
b. Entry and exit information to allow for censor will be created.
c. Using register based information as well as medical records, death due to breast cancer will be determined.
d. Exposure information including type of therapy will be assessed and categorized.

Summary:
1: Analysis using data from regional cancer register in Stockholm
We tested the hypothesis if women treated aggressively for their first breast cancer were more likely to die when diagnosed with a short latency metachronous cancer using a data from regional cancer register in Stockholm (Apendix 2 -Table 2).

Our results support the interpretation by showing a stage adjusted 2.4-fold higher mortality rate among women who received adjuvant chemotherapy following their first primary breast cancer, while there is no increased mortality following chemotherapy after the second primary cancer. We believe that the findings support a selection process where adjuvant systemic treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically.

2: Analysis using data collected from medical records
Our aim was to investigate first if the higher mortality could be confirmed by a higher incidence of distant metastasis and second if the worsen prognosis could be explained by existing tumour characteristics or by copy number aberrations in the tumour genes. We included a population based cohort of 339 contralateral breast cancer patients diagnosed within 5 years of primary breast cancer between 1975-2005 in Stockholm, Sweden.

Our cohort for analysis of possible therapeutic resistance in short latency bilateral cancer comprised 17,089 women, among these women, 441 developed metachronous bilateral disease within 5 years of their primary breast cancer during follow-up through 2005. We included only women with TNM stage 1-3 at first diagnosis in order to minimize the risk of misclassified

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metastatic disease and further to minimize confounding by indication for adjuvant treatment in relation to bilateral breast cancer death. For all these women all medical records were retrieved and information on treatment, tumor characteristics, hormonal information etc were ascertained and computerized. To date 97% of 392 women with metachronous cancers diagnosed within 5 years have been retrieved.

The occurrence of distant metastasis within 5 years was used as a measure of bad prognosis and was ascertained from the Regional Oncological Center, Stockholm as well as the medical records. Incidence rate of distant metastasis was calculated with the accumulated person-time at risk as the denominator. This time started at second diagnosis for bilateral breast cancer and continued until occurrence of distant metastasis, emigration, death or end of follow-up (December 31, 2005), whichever came first. Poisson regression was used for modeling of occurrence of distant metastasis. Analysis adjusted for time since second diagnosis, calendar period, stage of first breast cancer, age, stage and treatment of second cancer.

Contralateral breast cancer patients receiving adjuvant therapy for their first cancer have an increased risk of metastasis after their second cancer (Table 1). The risk increase seems to be most pronounced for chemotherapy. To assess bias by indication (i.e. the aggressive cancers get more adjuvant therapy) we analyzed risk for metastasis by adjuvant therapy for the second cancer as well, and found no indication that this therapy affected the risk (data not shown).

Further we investigated if the poor prognosis is conveyed through known prognostic tumour characteristics. Table 2 shows that adjuvant therapy might increase the risk that the second tumour compared to the first is of worse TNM stage and Elston grade, though the findings are non-significant.

Table 1: Incidence Rate Ratio of distant metastasis after contralateral breast cancer, by treatment of first primary breast cancer.

Treatment groups	IRR	(Confidence Interval 95%)
Any adjuvant therapy	2.37	(1.31 – 4.30)
Radiotherapy	2.58	(1.35 – 4.91)
Hormone therapy	1.39	(0.62 – 3.08)
Homone therapy and chemotherapy	2.54	(0.91 – 7.17)
Chemotherapy	3.48	(1.60 – 7.5)
No adjuvant therapy	1.00	(Ref.)

Table 2: Risk of having more aggressive tumour characteristics at second breast cancer compared to first; adjuvant therapy vs. no adjuvant therapy (ref)

Tumour characteristics	Odds Ratio (95% CI)
TNM Stage (1, 2, 3)	1.79 (0.74 – 4.33)
Elston-Ellis Grade (1, 2, 3)	1.94 (0.69 – 5.49)
Multifocal (multifocal, non-multifocal)	0.80 (0.34 – 1.90)
Size (<2mm, 2-5mm, >5mm)	0.88 (0.32 – 2.40)

Task 4. Analysis of tissue, Months 6-7:

a. A quality assurance protocol will be instituted to check the specimen quality, n=50+50.
b. Fluorescence in situ hybridization analysis (QM-FISH) will setup and standardized.
c. Analysis of gene copy number (amplification and deletion) with array technology will be setup and standardized.

Summary:
Our focus was first on the setup and standardization of QM-FISH method. QM-FISH (Quantitative Multi-gene Fluorescence In-Situ Hybridization) developed by Anders Zetterberg's lab that was used to study gene copy number changes (allelic imbalances) in about 50 selected genes. Starting from the conventional FISH technique, he has developed a quantitative, multi-gene technique which enables us to study gene copy number changes (allelic imbalances) as can be seen in Figure 1. In a larger study conducted by Zetterberg et al., genetic rearrangements were identified that are associated with worse survival in breast cancer patients, even after accounting for tumor grade, ER status, progesterone status, node status and tumor size [7].

As a result of methodological development in Anders Zetterberg's lab, several individual genes can now be identified and quantified accurately and reproducibly in each tumor cell nucleus at the same time which makes the technique suitable for large-scale clinical studies. The technique consists of new protocols for hybridization, 3D-fluorescence microscopy with a Z-scanning devise, a CCD-camera for image acquisition and PCs for control functions and image analysis. The digital image analysis algorithms and software that have been developed at Karolinska Institutet consists of an optimized combination of existing as well as new powerful algorithms. This has resulted in a QM-FISH-technique with very high sensitivity and specificity. Technique has been developed to generate QM-FISH-probes from any region of the genome. With the current methodology, the influence of FISH-artefacts (loss of signals and/or split signals) has been reduced and gene copy number can be accurately quantified in over 90% of the cells in a cell population.
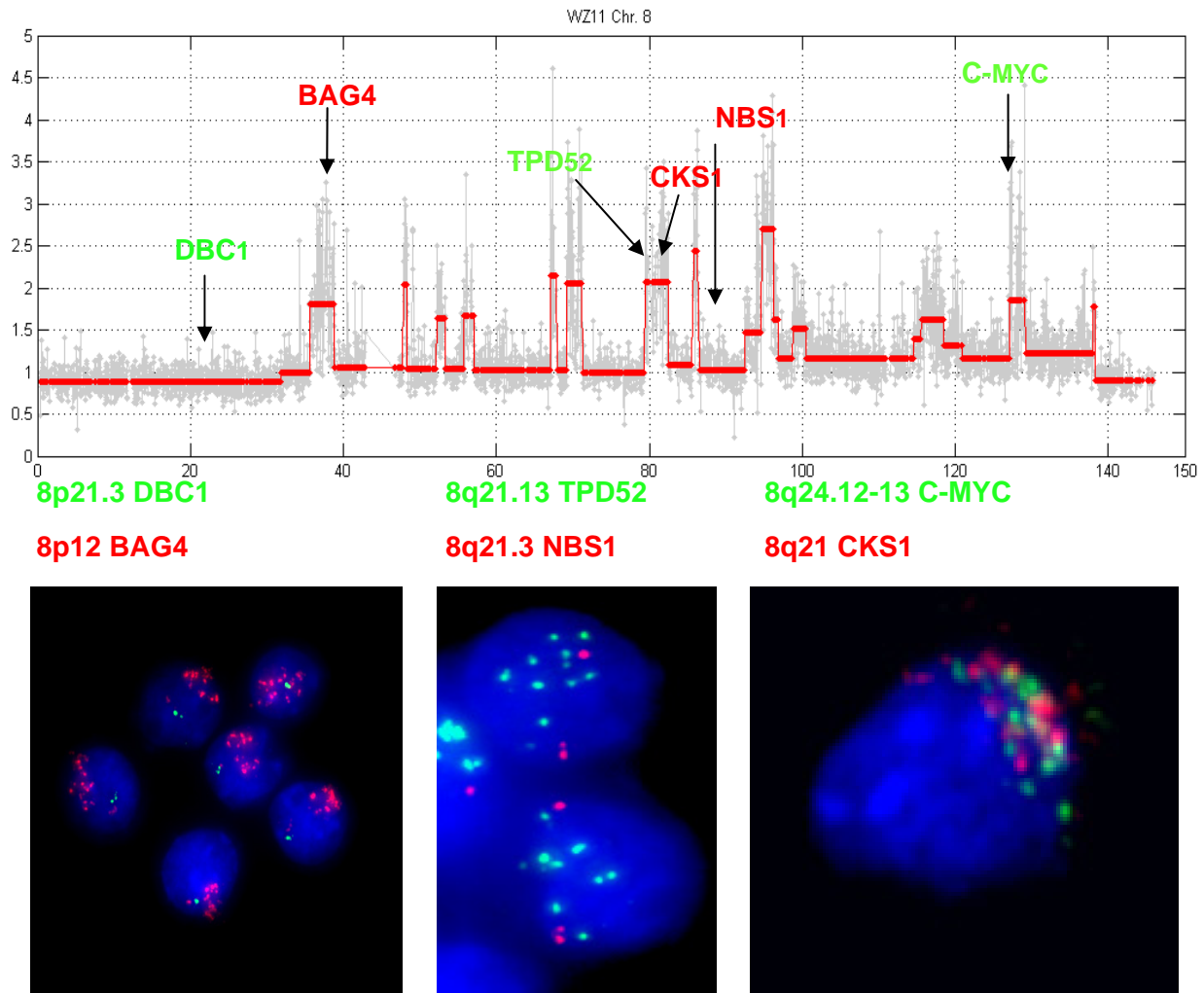
Figure 1. QM-FISH has previously been used to validate amplifications and deletions identified in breast cancers by interphase FISH. Aberrations on chromosome 8, amplifications (*e.g.* BAG4) and deletions (*e.g.* DBC1), are clearly displayed by QM-FISH in the lower left-hand panel.

Task 5. Analysis of 50 bilateral cases and 50 controls. Months 8-9:

a. The data analysis will be detailed and analysis of the final data set will be conducted using SAS statistical software package

Summary:
Information from the medical records were used to do the statistical analysis and for selecting patients for the molecular analysis QM- FISH. Since the collection of tissue samples was problematic due to the new regulations in Sweden we were able to collect and analyze only 22 tissue samples. To compensate for this we included an additional study (not included in the original proposal) using collection of detailed information on tumor characteristics from medical

records (results summarized in Table 2). We also focused on more thorough improvement and standardization of molecular methods (results demonstrated in Figure 1).

By representational oligonucleotide microarray analysis (ROMA) of breast cancer samples we have been able to identify the most amplified and the most often deleted regions in the genome. In order to get higher resolution we proceeded to produce probes for Quantitative Multicolor FISH (QM-FISH) method for 14 selected genes (Table 3) in the amplified regions. Five of the genes are marked with a single color, and 9 genes are marked with two colors in such close proximity that they appear to be on the same place when the samples are analyzed. To be able to do a full analysis four different areas in each tissue slides are photographed, every area is photographed in 25 layers so that no signal can be missed. The photographs are analyzed on a computer screen, the amplifications are easily visible for the eye but we have discovered that the deletions are very time consuming to analyze, and a computer program needs to be constructed for aiding in the analyzes. The analyzing personnel were naturally blinded to the exposed/unexposed status of the samples.

From earlier trials with QM-FISH we have come to expect that around 30% of the breast cancer cells have amplifications in the marked genes and our findings was in accordance with this. 11 exposed and 11 unexposed patients were analyzed, the exposed patients are defined as having their cancers separated with between three months and five years and being treated with chemotherapy, the unexposed have the same latency period and have not been treated with chemotherapy, the group was frequency matched on age (above/below 50), estrogen receptor status and latency between cancers (per year). We found amplifications in 4 exposed and 5 unexposed patients (Table 4). For the exposed samples there were amplifications in Cyklin D (two samples), cerbB2, CTTM, C-myc, Cks1a, CCNE2 and possibly in IgF 1R and the unexposed had amplifications in cerbB2 (2 samples), IgF 1R (two samples), Cyklin D, CTTM and MDM2. We do not see any trend regarding the frequency of amplifications nor the gene amplified in the exposed group vs. the unexposed group.

Table 3: 14 selected genes for QM-FISH

Amplified gene	Marker
C-myc	Green
Cyclin D	Orange
CTTN	Blue
cerbB2	Red
PIC3CA	Infra Red
MDM2	Green-Orange
IGF1R	Green-Red
BAG4	Green-Infra Red
Cks1a + CCNE2	Green-Blue
STK6	Orange-Red
INTS4	Orange-Infra Red
₉BCAS3	Red-Infra Red
NGFR	Red-Blue

Table 4: Amplifications analyzed using QM-FISH

| Exposure status	Sample number	Areas 1	2	3	4	Back-up	
Chemotherapy	1	NAD	NAD	NAD	NAD		
Chemotherapy	2	A	A	A	A		Cyklin D and CTTN amplifed, IgF1R
Chemotherapy	3	NAD	NAD	NAD	NAD		
Chemotherapy	4	NAD	NAD	NAD	NAD		
Chemotherapy	5	NAD	NAD	NAD	NAD		
Chemotherapy	6	NAD	NAD	NAD	NAD		
Chemotherapy	7	NAD	NAD	NAD	NAD		
Chemotherapy	8	NAD	NAD	NAD	NAD		
Chemotherapy	9	A	A	A	A		CycD amplifed
Chemotherapy	10	NAD	A	A	A		cerbB2 amplified
Chemotherapy	11	A?	A	A	NAD		C-myc, Cks1a and CCNE2 amplified
No Chemotherapy	12	NAD	A	A	A		Cyklin D and CTTN amplified.
No Chemotherapy	13	A	A	A	A		cerbB2 and INGFR amplified
No Chemotherapy	14	NAD	NAD	NAD	NAD		
No Chemotherapy	15	A	A	A	A		INGFR amplified
No Chemotherapy	16	NAD	NAD	NAD	NAD		
No Chemotherapy	17	NAD	NAD	NAD	NAD		
No Chemotherapy	18	NAD	NAD	NAD	NAD		
No Chemotherapy	19	NAD	NAD	NAD	NAD		
No Chemotherapy	20	NAD	NAD	NAD	NAD		
No Chemotherapy	21	NAD	NAD	A?	A	A	cerbB2 amplified
No Chemotherapy	22	A?	A	A?	A		MDM2 amplifed
						A =	amplification
						NAD =	no amplification detected
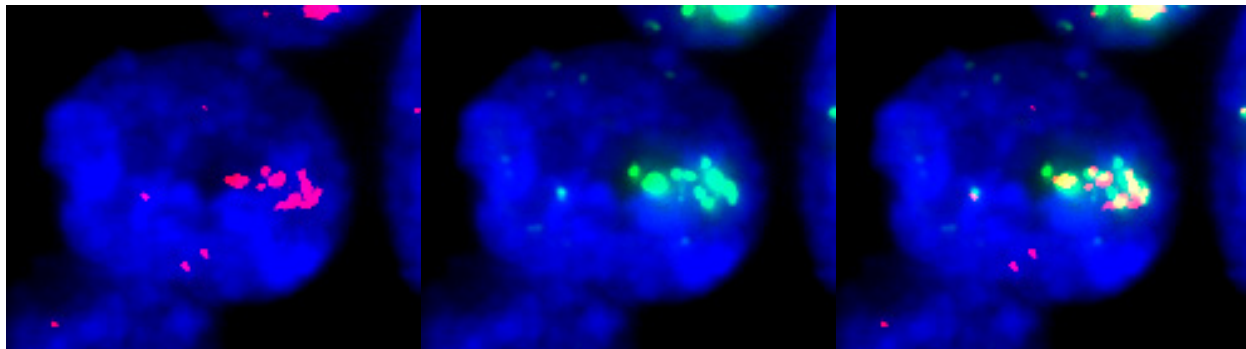
Figure 2. Example of a cell from sample 2, where Cyklin D and CTTN are amplified. Red marks CTTN and green Cyklin D. On the third picture both are displayed simultaneously, yellow is used to mark that both colors are on the same spot, which usually occurs when the chromosome configuration is correct, since both genes are situated on chromosome 11q

Task 6. Report and manuscript preparation, Months 10-12:

a. Manuscripts will be prepared.
b. A final report of the findings will be written.

Summary:
One manuscript has been written using our results from register-based studies, see Appendix 2. One more manuscript is in preparation based on data collected from medical records. We are planning the third manuscript on molecular studies of prognosis of bilateral breast cancers with short latency.

3. Key research accomplishments

1) Identification of metachronous bilateral breast cancers and collection of data for 97% of cases.
2) Analysis of data collected from medical records showing that adjuvant treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically.
3) Analysis of tissue samples using newly improved QM-FISH technique.

4. Reportable outcomes

1) Article: Hartman M. Czene K. Reilly M. Adami H-O Dickman P.W. and Per Hall P. (2007) Incidence and prognosis of synchronous and metachronous bilateral breast cancer, Journal of Clinical Oncology, 25(27):4210-6.

2) Dissertation, Mikael Hartman, Thesis: Risk and prognosis of breast cancer among women at high risk of the disease, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, October 12, 2007

3) Abstract: Incidence and prognosis of synchronous and metachronous bilateral breast cancer, The 29th Annual San Antonio Breast Cancer Symposium, December 14 - 17, 2006 in San Antonio, Texas.

5. Conclusion

Contralateral breast cancer patients receiving adjuvant therapy for their first cancer have an increased risk of metastasis after their second cancer. The risk increase seems to be most pronounced for chemotherapy. Adjuvant therapy might increase the risk that the second tumour compared to the first is of worse TNM stage and Elston grade, though the findings are non-significant.

6. References

1.   Ries LAG EM, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Feuer EJ, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2001. Bethesda, MD, National Cancer Institute, 2004

2.	Robbins GF, Berg JW: Bilateral Primary Breast Cancer; a Prospective Clinicopathological Study. Cancer 17:1501-27, 1964

3.	Chen Y, Thompson W, Semenciw R, et al: Epidemiology of contralateral breast cancer. Cancer Epidemiol Biomarkers Prev 8:855-61, 1999

4.	Chen Y, Semenciw R, Kliewer E, et al: Incidence of second primary breast cancer among women with a first primary in Manitoba, Canada. Breast Cancer Res Treat 67:35-40, 2001

5.	Robinson E, Rennert G, Rennert HS, et al: Survival of first and second primary breast cancer. Cancer 71:172-6, 1993

6.	Holmberg L, Adami HO, Ekbom A, et al: Prognosis in bilateral breast cancer. Effects of time interval between first and second primary tumours. Br J Cancer 58:191-4, 1988

7.	Hicks J, Krasnitz A, Lakshmi B, et al: Novel patterns of genome rearrangement and their association with survival in breast cancer. Genome Res 16:1465-79, 2006

7. Appendices

1) Abstract form for data collection from medical records
2) Hartman M. Czene K. Reilly M. Adami H-O Dickman P.W. and Per Hall P. (2007) Incidence and prognosis of synchronous and metachronous bilateral breast cancer, Journal of Clinical Oncology, 25(27):4210-6.

APPENDIX 1

Uppdaterad: 2007-4-20 /MS

RABBIT

- Bilateral Breast cancer
studied from patient Information and Tissue

Abstract form -metachronous

Patient Sequence No.

Registered in the data base

Exkluderad

- -

Date of abstraction:
Code: yyyy-mm-dd

Abstractor:
Code: abstractors initials

First diagnose date:
Code: yyyy-mm-dd

Second diagnose date:
Code: yyyy-mm-dd

- -

Notes:

Patient information at time for first breast cancer diagnosis

Socio-economical status

Profession

Relevant earlier diseases

Earlier benign breast disease: ☐ Code: 1= yes, 2= no, 998= unknown

Diabetes: ☐ Code: 1= yes, 2= no, 998= unknown

Thyroid disease: ☐ Code: 1= yes, 2= no, 998= unknown

Schizophrenia ☐ Code: 1= yes, 2= no, 3=other psychiatric illness, 998= unknown

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Smoking info (at time of first breast cancer diagnosis)

Smoker ☐ Code: 1= current, 2= no, 998= unknown

If no: ☐ Code: 1= never, 2 = previous smoker, 998= unknown,

Hormone related info

Menarche ☐ Code: age or 998= unknown

Pregnancy ☐ Code: 1=yes, 2= no, 998= unknown

If yes, give number ☐ Code: number, 998=unknown,

Parity ☐ Code: 1=yes, 2= no, 998= unknown

If yes, give number ☐ Code: number, 998=unknown,

First partus ☐ Code: age, 998= unknown,

Breast feeding ☐ Code: 1= yes, 2= no, 998= unknown,

Menopause *at time of breast cancer diagnosis* ☐ Code: 1= yes, 2= no, 998= unknown

Menopause, age ☐ Code: age or 998= unknown,

Oral contraceptive *at time of breast cancer diagnosis* ☐ Code: 1= current, 2= no, 998= unknown

If no; ☐ Code: 1= never, 2 = previous, 998= unknown,

Hormone replacement Therapy at time of breast cancer diagnosis ☐ Code: 1= current, 2= no, 998= unknown

RABBIT - Risk factors for Acquiring Bilateral Breast cancer studied from patient Information and Tissue

If no; [ ]   Code: 1= never, 2 = previous, 998= unknown,

Anthropometric measures

Height: [ ]   Code: height in meters or 998 = unknown

Weight: [ ]   Code: weight in kilos or 998 = unknown

Build/physique: [ ]   Code: 1= overweight, 2= normal, 3=underweight, 998= unknown

---

Heredity (at time of first breast cancer diagnosis)

Family history of breast cancer [ ]   Code: 1= yes, 2= no, 998= unknown

Family history of ovarian cancer [ ]   Code: 1= yes, 2= no, 998= unknown

Relationship [ ]   Code: 1= parent, 2= sibling, 3= offspring, 4= more distant, 998=unknown,

Type of cancer [ ]   Code: 1= breast, 2= ovary, 3= both,

Relationship [ ]   Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer [ ]   Code: 1= breast, 2= ovary, 3= both,

Relationship [ ]   Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer [ ]   Code: 1= breast, 2= ovary, 3= both,

Relationship [ ]   Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer [ ]   Code: 1= breast, 2= ovary, 3= both,

First Breast cancer

Date of diagnosis [ ]   Code: yyyy-mm-dd or 998= unknown

Location [ ]   Code: 1= right, 2= left, 3= bilateral, 998= unknown

PAD no. [ ]   Code: Actual number, 998=unknown, 777=PAD do not exist

PAD report copied [ ]   Code: 1= yes, 2= no,

Mode of detection [ ]   Code: 1= palpation by patient, 2= screening mammography, 3= palpation by MD,
                                           4= control mammography, 5=other, 998= unknown

If other; name it [ ]

Surgical info - First Breast cancer

Surgery [ ]   Code: 1= yes, 2= no, 998= unknown

RABBIT - Risk factors for Acquiring Bilateral Breast cancer studied from patient Information and Tissue

Surgery, date _____ Code: yy-mm-dd or 998= unknown,

Surgery, type _____ Code: 1= partial mastectomy, 2= total mastectomy,
 3= biopsy, 998= unknown,

Lymph nodes examined _____ Code: 1= yes, 2= no, 998= unknown

If yes, positive findings _____ Code: 1= yes, 2= no, 998= unknown,

Number of positive lymph nodes _____ out of _____ examined lymph nodes

Code: actual number, 998= unknown,

Periglandular growth _____ Code: 1= yes, 2= no, 998= unknown

Tumor characteristics -First breast cancer

Information source _____ Code: 1= PAD/ tissue analysis, 2= cytology, 998= unknown,

Histology _____ Code: 1= ductal, 2= lobular, 3= other, 998= unknown

If other (=3), specify _____

Multiple tumors _____ Code: 1= yes, 2= no, 998= unknown

Further comments _____

Largest tumor size _____ Code: size in mm (given in PAD) or 998= unknown

Tumor cell differentiation _____ Code: 1= high, 2= intermediate, 3= low, 998= unknown

Estrogen receptor status _____ Code: 1= positive, 2= negative, 3=positive, unclear laterality,
 4= negative, unclear laterality 998= unknown

Value: _____ Code: value _____ Code: unit

Progesterone receptor status _____ Code: 1= positive, 2= negative, 3=positive, unclear laterality,
 4= negative, unclear laterality 998= unknown

Value: _____ Code: value _____ Code: unit

S-phase _____ % Code: value in percent or 998=unknown

Treatment -First Breast cancer

Radiotherapy (RT) _____ Code: 1= yes, 2= no, 998= unknown

RT, type _____ Code: 1= preoperative, 2= postoperative , 3= not surgically treated
 998= unknown

RT, start date _____ Code: yy-mm-dd or 998= unknown,

4

RABBIT - Risk factors for Acquiring Bilateral Breast cancer studied from patient Information and Tissue

Chemotherapy (CT) neoadjuvant [ ]   Code: 1= yes, 2= no, 998= unknown

CT used [ ]   Code: 1= FEC/FAC, 2=Taxotere, 3= CMF, 4= other, 998= unknown,

      If other, specify: [ ]

CT, start date [ ]   Code: yy-mm-dd, 998=unknown ,

Duration of CT [ ]   Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

Adjuvant chemotherapy (CT) [ ]   Code: 1= yes, 2= no, 998= unknown

CT used [ ]   Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

      If other, specify: [ ]

CT, start date [ ]   Code: yy-mm-dd, 998= unknown,

Duration of CT [ ]   Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

Adjuvant hormone therapy (HT) [ ]   Code: 1= yes, 2= no, 998= unknown

HT used [ ]   Code: 1= Tamoxifen only, 2=other, 998= unknown,

      If other (=2), specify [ ]

Dose of HT [ ]   Code: daily dose (in mg), 998= unknown,

HT, intended time of use [ ]   Code: months, 998=unknown,

- -

Recurrent tumors -First breast cancer

New, local or regional ipsilateral tumor [ ]   Code: 1= yes, 2= no, 998= unknown,

Type of recurrent tumor [ ]   Code: 1=local, 2=regional, 998=unknown, 222=not applicable

Date of diagnosis [ ]   Code: yy-mm-dd,

Surgery, recurrent tumor [ ]   Code: 1=yes, 2=no, 998=unknown,

PAD no. [ ]   Code: Actual number, 998=unknown, 777=PAD do not exist

PAD report copied [ ]   Code: 1= yes, 2= no,

RT, recurrent tumor [ ]   Code: 1= yes, 2= no, 998= unknown,

CT, recurrent tumor [ ]   Code: 1= yes, 2= no, 998= unknown,

RABBIT - Risk factors for Acquiring Bilateral Breast cancer studied from patient Information and Tissue

CT Used [   ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [   ]

CT, start datum [   ] Code: yy-mm-dd, 998= unknown, 222=not applicable

Duration of CT [   ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [   ]

HT, recurrent tumor [   ] Code: 1= yes, 2= no, 998= unknown ,

- -

Second breast cancer

Date of diagnosis [   ] Code: yy-mm-dd or 998= unknown

Diagnosed during treatment [   ] Code: 1= yes, treatment for BCI, 2= no, 3=yes, treatment for recurrences

Laterality [   ] Code: 1= right, 2= left, 3= bilateral, 998= unknown

PAD no. [   ] Code: Actual number, 998=unknown,

PAD report copied [   ] Code: 1= yes, 2= no

Mode of detection [   ] Code: 1= palpation by patient, 2= screening mammography, 3= palpation by MD, 4= control mammography, 5=other, 998= unknown

If other; specify [   ]

Further comments [   ]

- -

Surgical info –second breast cancer

Surgery [   ] Code: 1= yes, 2= no, 998= unknown

Surgery, date [   ] Code: yy-mm-dd or 998= unknown,

Surgery, type [   ] Code: 1= partial mastectomy, 2= total mastectomy, 3= biopsy, 998= unknown,

Lymph nodes examined [   ] Code: 1= yes, 2= no, 998= unknown

If yes, positive findings [   ] Code: 1= yes, 2= no, 998= unknown,

Number of positive lymph nodes [   ] out of [   ] examined lymph nodes

Code: actual number, 998= unknown,

Periglandular growth [   ] Code: 1= yes, 2= no, 998= unknown

- -

Tumor characteristics –second breast cancer

Information source ⬚ Code: 1= PAD/ tissue analysis, 2= cytology, 998= unknown,

Histology ⬚ Code: 1= ductal, 2= lobular, 3= other , 998= unknown

If other (=3), specify ⬚

Multiple tumors ⬚ Code: 1= yes, 2= no, 998= unknown

Further comments ⬚

Largest tumor size ⬚ Code: size in mm (given in PAD) or 998= unknown

Tumor cell differentiation ⬚ Code: 1= high, 2= intermediate, 3= low, 998= unknown

Estrogen receptor status ⬚ Code: 1= positive, 2= negative, 3=positive, unclear laterality,
4 =negative, unclear laterality 998= unknown

Value: ⬚ Code: value ⬚ Code: unit

Progesterone receptor status ⬚ Code: 1= positive, 2= negative, 3=positive, unclear laterality,
4 = negative, unclear laterality 998= unknown

Value: ⬚ Code: value ⬚ Code: unit

S-phase ⬚ % Code: value in percent or 998=unknown

- -

Treatment -second breast cancer

Radiotherapy (RT) ⬚ Code: 1= yes, 2= no, 998= unknown

RT, type ⬚ Code: 1= preoperative, 2= postoperative , 3= not surgically treated
998= unknown

RT, start date ⬚ Code: yy-mm-dd or 998= unknown,

Chemotherapy neoadjuvant ⬚ Code: 1= yes, 2= no, 998= unknown

CT used ⬚ Code: 1= FEC/FAC, 2=Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: ⬚

CT, start date ⬚ Code: yy-mm-dd or 998= unknown,

Duration of CT ⬚ Code: Actual number of cycles, 888=continuous or 998=unknown,

Further comments ⬚

Adjuvant chemotherapy (CT) [ ] Code: 1= yes, 2= no, 998= unknown

CT Used [ ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start date [ ] Code: yy-mm-dd or 998= unknown,

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

Adjuvant hormone therapy (HT) [ ] Code: 1= yes, 2= no, 998= unknown

HT used [ ] Code: 1= Tamoxifen only, 2=other, 998= unknown,

If other (=2), specify [ ]

Dose of HT [ ] Code: daily dose (in mg), 998= unknown

HT, intended time of use [ ] Code: months, 998=unknown,

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Recurrent tumors -Second Breast cancer

New, local or regional ipsilateral tumor [ ] Code: 1= yes, 2= no, 998= unknown,

Type of recurrent tumor [ ] Code: 1=local, 2=regional, 998=unknown, 222=not applicable

Date of diagnosis [ ] Code: yy-mm-dd,

Surgery, recurrent tumor [ ] Code: 1=yes, 2=no, 998=unknown,

PAD no. [ ] Code: Actual number, 998=unknown, 777=PAD do not exist

PAD report copied [ ] Code: 1= yes, 2= no,

RT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT Used [ ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start datum [ ] Code: yy-mm-dd, 998= unknown, 222=not applicable

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

HT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

Distant metastasis after 2nd BC diagnosis [ ] Code: 1= yes, 2= no, 998= unknown

Date of met. [ ] Code: yy-mm-dd, 998= unknown,

Diagnosed during treatment for 2nd BC [ ] Code: 1= yes, 2= no, 998= unknown

Localization of first distant metastasis [ ] Code: 1 = lung, 2 = liver, 3 = skeleton, 4 = brain, 5 = other, 998= unknown,

If other, specify: [ ]

Last date of follow-up [ ]

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Additional PADs

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

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Hormone related info –second breast cancer

Menopause *at time of*
breast cancer diagnosis   [ ]   Code: 1= yes, 2= no, 998= unknown

Menopause, age   [ ]   Code: age or 998= unknown,

HRT *at time of*
breast cancer diagnosis   [ ]   Code: 1= current, 2= no, 998= unknown

If no;   [ ]   Code: 1= never, 2 = previous, 998= unknown,

- -

Mammography info *–closest to first breast cancer diagnosis*

 Mammography, copy of   -pictures   [ ]   Code: 1= yes, 2= no original available

                         -density scaling [ ]   Code: 1= yes, 2= no original available

Date of mammography: [   ]   Code yy-mm-dd (as close to first diagnosis as possible), 998=unknown

Second opinion of mammography pictures: [ ]   Code: 1= yes, 2= no, 998= unknown

                         Density scaling   [ ]   Code:

Comments

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Incidence and Prognosis of Synchronous and Metachronous Bilateral Breast Cancer

Mikael Hartman, Kamila Czene, Marie Reilly, Jan Adolfsson, Jonas Bergh, Hans-Olov Adami, Paul W. Dickman, and Per Hall

A B S T R A C T

Purpose
Because the incidence of breast cancer is increasing and prognosis is improving, a growing number of women are at risk of developing bilateral disease. Little is known, however, about incidence trends and prognostic features of bilateral breast cancer.

Patients and Methods
Among 123,757 women with a primary breast cancer diagnosed in Sweden from 1970 to 2000, a total of 6,550 developed bilateral breast cancer. We separated synchronous (diagnosed within 3 months after a first breast cancer) and metachronous bilateral cancer, and analyzed incidence and mortality rates of breast cancer using Poisson regression models.

Results
The incidence of synchronous breast cancer increased by age and by 40% during the 1970s, whereas the incidence of metachronous cancer decreased by age and by approximately 30% since the early 1980s, most likely due to increasing use of adjuvant therapy. Women who developed bilateral cancer within 5 years and at age younger than 50 years were 3.9 times (95% CI, 3.5 to 4.5) more likely to die as a result of breast cancer than women with unilateral cancer. Women with a bilateral cancer diagnosed more than 10 years after the first cancer had a prognosis similar to that of a unilateral breast cancer. Adjuvant chemotherapy of primary cancer is a predictor of poor survival after diagnosis of early metachronous cancers.

Conclusion
We found profound differences in the incidence trends and prognostic outlook between synchronous and metachronous bilateral breast cancer diagnosed at different ages. Adjuvant chemotherapy therapy has a dual effect on metachronous cancer: it reduces the risk, while at the same time it seems to worsen the prognosis.

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INTRODUCTION

Globally, increasing breast cancer incidence rates, improved prognosis, and growing life expectancy have resulted in increasing number of women at risk of developing a bilateral primary breast cancer. There are an estimated 2.2 million women living in the United States who have been diagnosed at some time with breast cancer.[1] Hence, optimal surveillance and clinical management of women who have had one or two primary breast cancers is a challenge. However, there are only limited data on incidence rates of synchronous and metachronous breast cancer,[2,3] results on temporal trends in incidence are conflicting,[4] and little is known about the prognostic outlook after treatment of a second primary cancer.[5,6]

We analyzed a large nationwide cohort of breast cancer patients in Sweden. We achieved complete follow-up with regard to incidence and survival during 1970 through 2000, a period when mammographic screening and adjuvant systemic treatment became broadly implemented. We also analyzed a separate cohort with detailed treatment information to understand better whether adjuvant chemotherapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer.

PATIENTS AND METHODS

Study Cohort

The study cohort was obtained from the nationwide Swedish Cancer Register, established in 1958 and estimated to be at least 98% complete.[7] For each notified cancer, the register includes the individually unique national registration number, the International Classification of Diseases code, and the date of diagnosis, but no

information on stage of disease or treatment. Using the national registration number, the Cancer Register can be linked to the nationwide Cause of Death Register and the Total Population Register. These linkages allow complete follow-up with regard to vital status, date and causes of death, as well as dates of immigration/emigration.

Because laterality of breast cancer was not recorded before 1970, we restricted the study cohort to the 138,372 women with a first primary invasive breast cancer diagnosed in the period 1970 to 2000. We excluded 8,123 women for whom the history of breast cancer was uncertain because they had immigrated to Sweden, and also excluded 6,492 women with a primary malignant tumor other than in the breast before the first breast cancer. Hence, our cohort for final analysis comprised a total of 123,757 women.

Validation Cohort

To take treatment and stage into consideration, we selected a validation cohort. Since 1976, all new primary breast cancers in the Stockholm-Gotland Health Care Region were reported to a central breast cancer register at the Regional Oncological Center, Stockholm.[8] The register holds information on stage, estrogen and progesterone receptor status, and adjuvant treatment. We identified 19,446 women with a first primary cancer. After excluding 2,357 women with a malignant tumor other than breast before the first breast cancer, our validation cohort for analysis comprised 17,089 women, of whom 952 developed contralateral breast cancer during follow-up through 1999. We included only women with TNM stage 1 to 3 at first diagnosis to minimize the risk of misclassified metastatic disease and also to minimize confounding by indication for adjuvant treatment in relation to bilateral breast cancer death. The validation cohort for final analysis comprised a total of 900 women with contralateral breast cancer and 16,320 women with unilateral cancer.

Statistical Analysis

Unilateral breast cancer incidence rates were calculated using Swedish female population counts as denominators. Bilateral cancers diagnosed within 3 months of the first primary were categorized as synchronous; the remaining cancers were categorized as metachronous. Because synchronous bilateral breast cancer was regarded as a simultaneous clinical event, the incidence rate was calculated as for unilateral breast cancer. The incidence rate of metachronous breast cancer was calculated using as the denominator the accumulated person-years at risk among women with unilateral breast cancer. The person-time at risk started 3 months after the date of first diagnosis and continued until diagnosis of bilateral breast cancer or of any other malignant disease, emigration, death, or end of follow-up (December 31, 2000), whichever came first.

Deaths as a result of breast cancer were ascertained from the Cause of Death Registry with high reported accuracy.[9] The mortality rate was calculated with the accumulated person-time at risk as the denominator. This time started at first diagnosis for unilateral and at second diagnosis for all bilateral breast cancer, and continued until diagnosis of bilateral cancer (for unilateral cancer), emigration, death, or end of follow-up (December 31, 2000), whichever came first. Any in situ breast cancer either before or after the first primary cancer was ignored.

We used the Nelson-Aalen method to estimate cause-specific cumulative mortality. Poisson regression was used for modeling of both bilateral breast cancer incidence and survival. We adjusted for age and calendar period of diagnosis in the incidence analysis with additional adjustment for time since diagnosis in the survival analysis. Within the validation cohort additional adjustment was made for TNM stage, estrogen receptor status, and adjuvant treatment. We present 5-year cause-specific mortality, with the exception of Nelson-Aalen cause-specific cumulative mortality, for which we present complete follow-up. We censored follow-up at age 80 years because classification of cause of death may be less reliable in older women.

All data preparation and analysis were done using the SAS Statistical package, version 8.2 (SAS Institute, Cary, NC).[10]

RESULTS

Incidence of Bilateral Breast Cancer

In the cohort of 123,757 women with a first breast cancer diagnosed between 1970 and 2000, a total of 6,550 women developed

synchronous (n = 1,893) or metachronous (n = 4,657) bilateral breast cancer during follow-up through 2000. Overall, approximately 1.6 synchronous cancers occurred per 10^5 person-years at risk. The incidence of synchronous cancer increased from 1970 until the mid-1980s and remained almost constant thereafter (Fig 1). The incidence
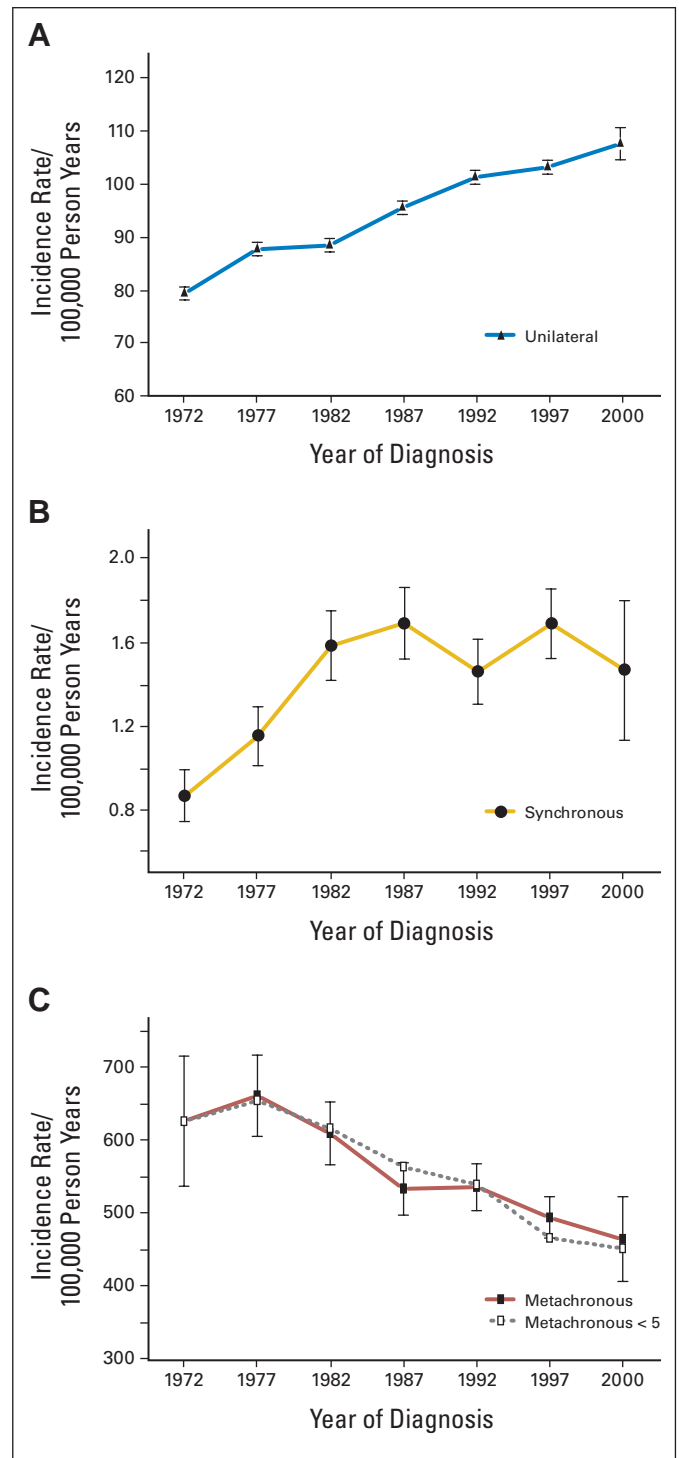

Fig 1. Temporal trends in incidence rates of (A) unilateral, (B) synchronous, and (C) metachronous bilateral breast cancer, in Sweden 1970 to 2000: 95% CIs are presented for all studied groups of breast cancer except metachronous cancer less than 5 years since primary tumor.
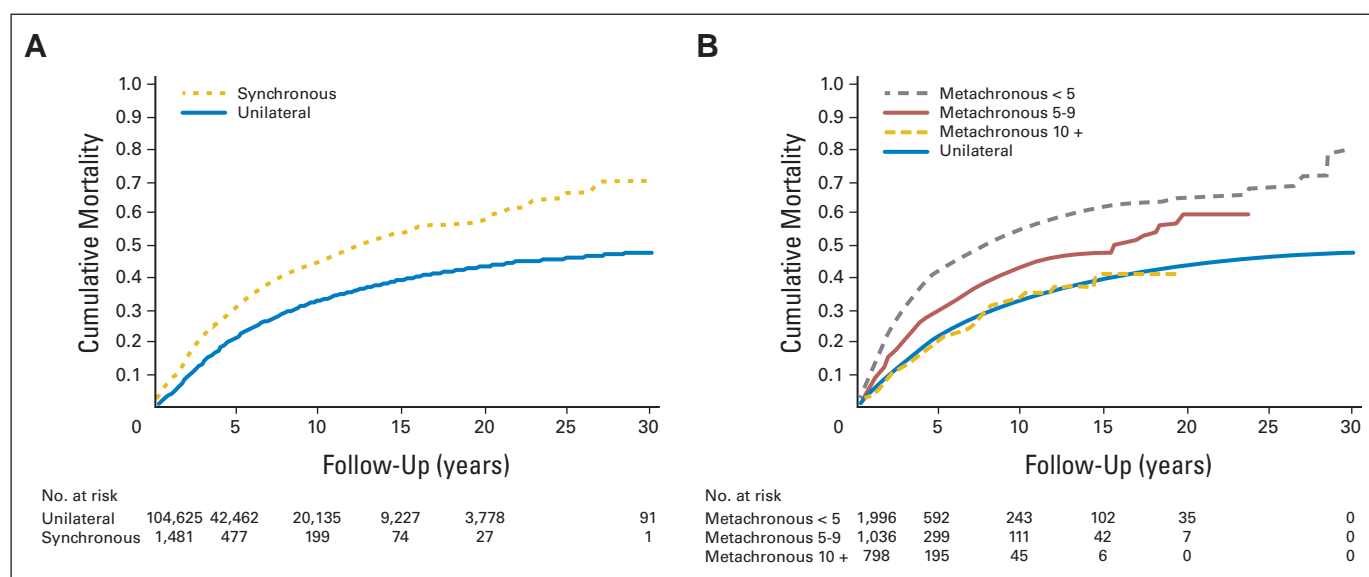
Fig 2. Nelson-Aalen estimates of breast cancer specific mortality after (A) unilateral, synchronous bilateral, and (B) metachronous bilateral breast cancer, stratified by time since diagnosis of unilateral breast cancer, with follow-up to age 80 years.

rate of metachronous cancer decreased by almost one third during the study period from 640 per 10^5 person-years at risk in 1970 to 440 per 10^5 person-years at risk in 2000. This overall decreasing trend was similar for metachronous cancers diagnosed within 5 years of the first primary breast cancer. In a multivariate Poisson regression model of bilateral breast cancer in relation to calendar period adjusted for age, we observe the same increasing trend of synchronous cancer as seen in Figure 1 (*P* for trend < .001; data not shown). The multivariate analyses of metachronous bilateral cancer limited to the first 5 years of follow-up revealed a similar and significant decreasing trend during the study period, as seen in Figure 1 (*P* for trend < .001).

Survival of Patients With Bilateral Breast Cancer

Women with synchronous bilateral breast cancer had a higher mortality from breast cancer than women with unilateral disease (*P* < .001; Fig 2A); after 10 years of follow-up, the cumulative breast cancer specific mortality was 45% (95% CI, 41.4% to 48.0%) and 33% (95% CI, 32.8% to 33.5%), respectively. Among women with meta-chronous breast cancer, the lowest mortality from breast cancer was seen for those with the longest time interval between the first and the second cancer (Fig 2B). After 10 years of follow-up, the cumulative breast cancer–specific mortality was 56% (95% CI, 53.0% to 58.5%) among women with bilateral cancer diagnosed within 5 years and 34% (95% CI, 28.6% to 39.8%) among those diagnosed with bilateral cancer more than 10 years after their first primary.

The 5-year breast cancer–specific mortality rate was only mod-estly related to age at diagnosis among women with unilateral disease (Fig 3A). After synchronous bilateral breast cancer, mortality de-creased from 136 per 10^3 person-years at age younger than 40 years to 73 per 10^3 person-years at age 70 to 79 years at diagnosis. The modi-fying effect of age was even more pronounced for metachronous bilateral breast cancer, with a more than three-fold gradient in mor-tality between women aged younger than 40 years at diagnosis (178 per 10^3 person-years) and those aged 70 to 79 years at diagnosis (55 per 10^3 person-years).
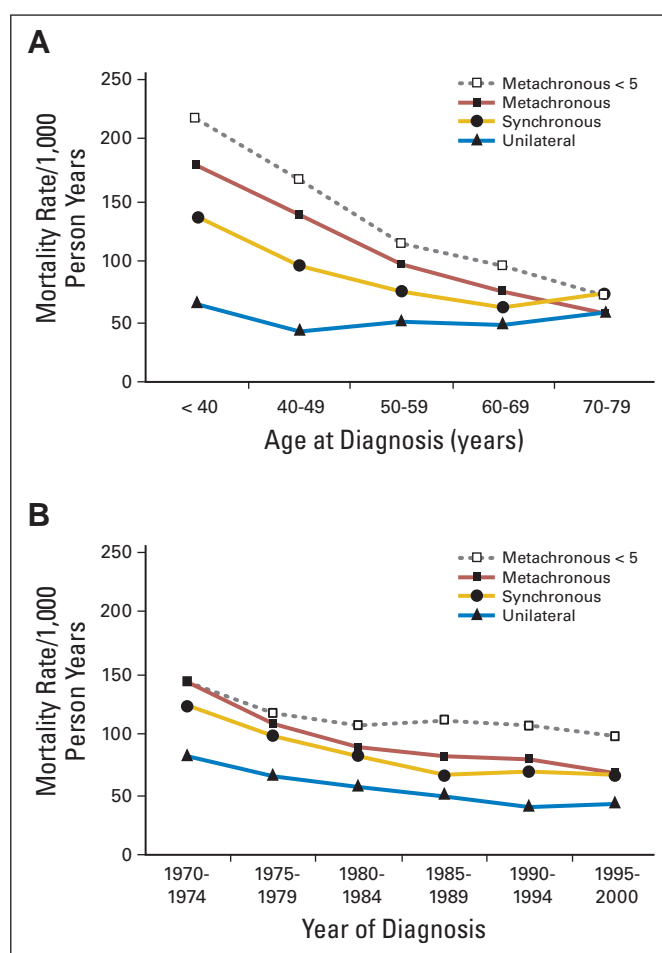

Fig 3. Breast cancer–specific mortality within 5 years after diagnosis of unilateral, synchronous bilateral, and metachronous bilateral breast cancer by (A) age and (B) period of diagnosis in Sweden 1970 to 2000 with follow-up to age 80 years.

The 5-year cause-specific mortality rate of synchronous cancer improved continuously during the study period from 124 per 10^3 person-years in 1970 to 1974 to 66 per 10^3 person-years in 1995 to 2000 (Fig 3B). Similarly, the 5-year cause-specific mortality rate of metachronous breast cancer improved during follow-up from 143 per 10^3 person-years to 68 per 10^3 person-years. This trend was less obvious for metachronous breast cancer diagnosed less than 5 years since unilateral breast cancer.

We used Poisson regression to estimate how mortality after bilateral breast cancer is affected by age at diagnosis of the first cancer and time interval to diagnosis of second breast cancer (Table 1). Compared with women with unilateral disease, those with synchronous bilateral cancer had a 40% higher mortality rate if they were 50 years or older, but had a 120% higher mortality rate if they were age < 50 years. Compared with women age < 50 years at unilateral breast cancer diagnosis, those who developed a metachronous cancer before 50 years of age within 5 years of their first breast cancer had an almost four-fold higher breast cancer mortality rate. This difference in prognosis between uni- and bilateral disease was reduced in older women and with longer time interval between the two cancers. Women with bilateral metachronous cancers diagnosed more than 10 years after initial diagnosis had a 5-year breast cancer

mortality not significantly different from that of women of the same age with a unilateral breast cancer.

Table 1 also lists results for the validation cohort with additional adjustment for TNM stage, estrogen receptor status (negative, < 0.05 fmol/μg DNA), and adjuvant treatment of primary cancer (for unilateral cancer) and second primary cancer (for bilateral cancer). Women with synchronous cancer had a 60% higher mortality rate compared with women with unilateral cancer. Women with metachronous cancer less than 5 years since primary had a more than four-fold higher mortality rate compared with women with unilateral cancer. Women with bilateral metachronous cancers diagnosed more than 10 years after initial diagnosis had a 5-year breast cancer mortality not significantly different from that of women of the same age with a unilateral breast cancer.

We next analyzed type of bilateral disease (synchronous v metachronous) and calendar time, stratified by age at first diagnosis, as determinants of survival (Fig 4A). Women age < 50 years diagnosed with synchronous breast cancer in 1970 to 1974 were approximately two times more likely to die as a result of breast cancer than women of the same age with unilateral breast cancer, a difference that varied only modestly over calendar period. In contrast, the excess death rate

Table 1. MRRs and 95% CIs From a Poisson Model of 5-Year Cause-Specific Mortality of Bilateral Breast Cancer Compared With Unilateral Breast Cancer Using the Predictors Age at Specified Diagnosis and Time Since Unilateral Breast Cancer Diagnosis

Age at Diagnosis (years)	Type of Cancer	Time Since Diagnosis of First Primary Cancer (years)	Deaths	MRR*†	95% CI
< 50	Unilateral‡		4,739	1.0 (ref)	
	Synchronous	< 0.25	77	2.2	1.8 to 2.8
	Metachronous	0.25-4	263	3.9	3.5 to 4.5
		5-9	52	2.4	1.8 to 3.2
		10-29	11	1.4	0.8 to 2.6
50-79	Unilateral‡		14,200	1.0 (ref)	
	Synchronous	< 0.25	278	1.4	1.2 to 1.6
	Metachronous	0.25-4	457	1.9	1.8 to 2.1
		5-9	189	1.5	1.3 to 1.7
		10-29	103	1.1	0.9 to 1.4
All ages	Unilateral		18,939	1.0 (ref)	
	Synchronous	< 0.25	355	1.5	1.4 to 1.7
	Metachronous	0.25-4	720	2.4	2.2 to 2.6
		5-9	241	1.6	1.4 to 1.9
		10-29	114	1.1	0.9 to 1.4
Validation cohort†					
All ages	Unilateral		1,713	1.0 (ref)	
	Synchronous	< 0.25	46	1.7	1.2 to 2.2
	Metachronous	0.25-4	98	4.2	3.4 to 5.3
		5-9	27	2.8	1.9 to 4.1
		10-29	9	1.0	0.5 to 2.0

Abbreviations: MRRs, mortality rate ratios; ref, reference.
*Adjusted for survival time, age, and calendar period of diagnosis.
†In a validation analysis, a subcohort of women with TNM stage 1-3 primary cancers from the Stockholm-Gotland Health Care Region was used. The validation cohort was adjusted for time since diagnosis, age at and calendar period of diagnosis, TNM stage, adjuvant treatment, oestrogen receptor status of primary cancer (for unilateral cancer) and second primary cancer (for bilateral cancer).
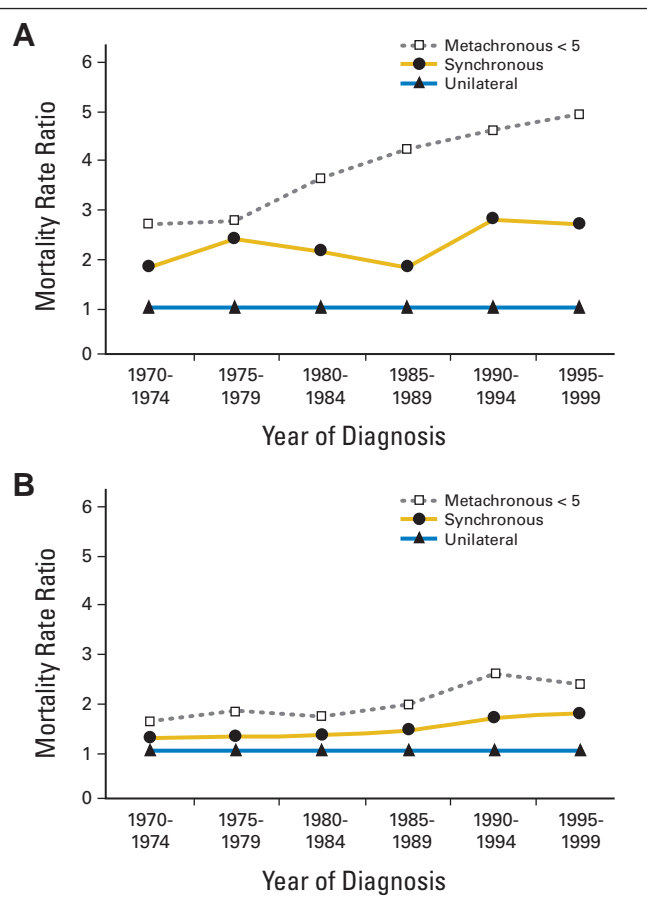‡Reference: unilateral breast cancer diagnosed at that age.

Fig 4. Poisson regression derived mortality rate ratios from a Poisson model estimating risk of breast cancer–specific mortality after unilateral, synchronous bilateral and metachronous bilateral breast cancer within 5 years of primary cancer by type of breast cancer, calendar period and (A) age < 50 years and (B) age 50 to 79 years at diagnosis of respective type of cancer with follow-up to age 80 years. Reference unilateral 5-year cause-specific mortality rate.

Table 2. MRRs and 95% CIs Obtained From a Poisson Model of 5-Year Cause-Specific Mortality Among Women Who Developed Metachronous Bilateral Disease Within 5 Years of Their Primary Breast Cancer in Relation to Adjuvant Treatment of Primary and Second Primary Cancer

Therapy	No. of Women	Type of Treatment*	No. of Deaths	MRR	95% CI
Therapy of first cancer†					
TNM stage 1-3‡	171	No chemotherapy	50	1.0 (ref)	
	47	Chemotherapy	27	2.4	1.3 to 4.4
TNM stage 1-2‡	150	No chemotherapy	34	1.0 (ref)	
	37	Chemotherapy	19	2.2	1.1 to 4.6
Therapy of second cancer§					
TNM stage 1-3‡	130	No chemotherapy	32	1.0 (ref)	
	50	Chemotherapy	10	1.2	0.5 to 2.9
TNM stage 1-2‡	119	No chemotherapy	22	1.0 (ref)	
	45	Chemotherapy	9	1.1	0.4 to 2.8

NOTE. Data from the Stockholm-Gotland Health Care Region.
Abbreviations: MRRs, mortality rate ratios; ref, reference.
*Chemotherapy is defined as exposed to systemic adjuvant chemotherapy with/without hormonal therapy and radiotherapy. No chemotherapy is defined as never exposed to systemic adjuvant chemotherapy.
†Adjusted for time since diagnosis, age, and calendar period of diagnosis, TNM stage of first and second cancer, estrogen receptor status of first and second cancer, and adjuvant treatment of second cancer.
‡TNM stage at primary diagnosis.
§Adjusted for time since diagnosis, age, and calendar period of diagnosis, TNM stage of first and second cancer, estrogen receptor status of first and second cancer, and adjuvant treatment of first cancer.

among women age $\leq$ 50 years with metachronous disease increased from less than three-fold to about five-fold during the study period. For women older than 50 years of age at diagnosis of breast cancer, the mortality rate ratio increased steadily from 1.2 to 1.7 (Fig 4B).

Finally, we analyzed the validation cohort to assess the effect of adjuvant treatment on breast cancer mortality among women diagnosed with metachronous cancer within 5 years of primary cancer (Table 2). Women who had received adjuvant chemotherapy after their first diagnosis were at 2.4-fold higher risk of death as a result of breast cancer compared with women who had received no such treatment when we adjusted for follow-up time, age, calendar period, TNM stage, hormone receptor status, and adjuvant treatment of the second cancer. Analysis of the mortality in women with TNM stage 1 to 2 at primary diagnosis revealed a similar significant two-fold excess mortality rate after adjuvant chemotherapy of primary cancer. For comparison, we calculated the mortality in women with bilateral disease depending on if they received adjuvant chemotherapy for the second primary cancer (Table 2). We observed no excess mortality in women subjected to adjuvant chemotherapy after the second primary cancer. In addition, we observed that women diagnosed with a first primary TNM stage 2 and 3 cancer were at a two-fold (mortality rate ratio [MRR], 1.9; 95% CI, 1.0 to 3.9) and five-fold (MRR, 5.4; 95% CI, 2.4 to 12.1) increased risk of death as a result of the disease, respectively, compared with women with stage 1 first primary cancer. In comparison, women diagnosed with a second primary TNM stage 2 and 3 were at a three-fold (MRR, 2.6; 95% CI, 1.4 to 5.1) and seven-fold (MRR, 7.2; 95% CI, 3.3 to 15.8) increased risk of death as a result of the disease, respectively, compared with women with stage 1 first primary cancers.

DISCUSSION

We analyzed the occurrence pattern and prognosis of bilateral breast cancer and found marked differences between synchronous and metachronous cancer during the 30-year period of our study. The incidence pattern of synchronous cancer is similar to that of unilateral disease, although without any notable trends in recent decades. Metachronous disease, on the other hand, was much more common in younger patients and incidence rates declined steadily from approximately 1980, most likely due to the expanding use of adjuvant systemic therapy.[11] Striking features of the survival analyses included the much higher excess mortality after metachronous than synchronous disease and among younger than older women. When women with metachronous disease were compared with those with unilateral disease, the excess mortality increased markedly during calendar time and was remarkably influenced by time since first breast cancer. The excess mortality of metachronous disease seems to be due in part to treatment of the primary cancer.

Strengths of our study include the large size, the population-based prospective design, the possibility to define laterality, and the completeness of follow-up. Misclassification of metastatic disease as a second primary breast cancer is generally considered a smaller problem and could not explain the pattern of these findings. Indeed, there is no evidence that such misclassification, if it exists, would change during calendar time or differ by age.[12] Admittedly, the prognosis of bilateral breast cancer is difficult to assess because it is conditional on age and the woman surviving her first malignancy; deaths cannot be attributed unequivocally to first or second cancer.

The gradual increase in the incidence of synchronous disease during the 1970s coincides with the introduction of routine and bilateral mammography as part of the diagnostic work-up in women with unilateral cancer.[13] Such work-up may entail that some preclinical bilateral cancers are detected early and classified as synchronous disease[14] (perhaps in an earlier and more favorable stage[15]) rather than diagnosed later as metachronous disease.[16] The overall incidence rate of metachronous bilateral cancer in our study is also compatible with previous reports (Fig 1).[3,4,17-19] During the period of our study, adjuvant systemic therapy, mainly tamoxifen and chemotherapy, became

clinical practice. Because such treatment reduces the incidence of local recurrences, bilateral cancer, and distant metastasis,[11,20-22] it likely explains the substantial reduction in the incidence of metachronous bilateral disease during calendar time. Similar trends have been found in the United States[23] but not in Canada.[4]

Women diagnosed with unilateral cancer early in life and bilateral cancer within 5 years had a four times higher mortality rate than women with unilateral breast cancer after adjustment for age at diagnosis and calendar period (Table 1). The pattern persisted but the effect of time since first cancer was weaker in older age groups. In contrast, we observed that women with metachronous cancers diagnosed more than 10 years after initial diagnosis had a prognosis similar to that of a woman with unilateral cancer. This mortality pattern for women with bilateral cancer became even stronger after adjustment of stage and adjuvant treatment in the validation cohort. It is indeed notable that women with metachronous cancer diagnosed within 5 years after unilateral cancer have a higher mortality rate than women with synchronous bilateral cancer. Our findings are supported by previous studies,[6,24] although there are studies detecting no increased mortality for bilateral disease.[5,25] These differences may be attributed to variations in sample size, age, follow-up, and treatment regimes.

We also found that compared with women with unilateral disease, the prognostic outlook among women with metachronous disease deteriorated over time concomitantly with the decreasing incidence (Fig 4). This novel finding suggests that adjuvant systemic treatment selectively prevents the occurrence of cancers with a favorable prognosis, allowing those with a more aggressive phenotype to surface clinically. Adjuvant chemotherapy is administered more often to premenopausal women, whereas antiestrogens have been the primary choice among older women. Thus, the much stronger increase in mortality over time among women with bilateral cancer younger than age 50 years compared with women with unilateral disease suggests that chemotherapy exerts a stronger selection pressure than adjuvant endocrine treatment. Results from our validation cohort supported this interpretation by showing a stage-adjusted 2.4-fold higher mortality rate among women who received adjuvant chemotherapy after their first primary breast cancer (Table 2).

One explanation for this finding would be that chemotherapy is administered to women with more aggressive tumors and hence with poorer survival. Conversely, excess mortality after chemotherapy of primary cancer was also observed for women with TNM stage 1 to 2 cancer, whereas we observed no increased mortality among women treated with adjuvant chemotherapy after the second primary cancer. We believe that the findings support a selection process of more malignant second primary cancers, given the bias by indication for adjuvant chemotherapy should be similar for the choice of treatment of primary and second primary cancer. The result of the validation cohort thus suggests that such bias is minor.

Our findings also may be relevant for clinical management of women with breast cancer. It is not surprising that women diagnosed with two simultaneous cancers have an increased mortality compared with women with one cancer. What is puzzling is the strong age dependency of this effect. Perhaps even more challenging to explain is the remarkable excess mortality among women who develop short latency metachronous disease at young ages. There is no obvious reason why there should be a difference in biologic behavior between the first and second tumor because the breast tissue is influenced uniformly by the same genetic and environmental factors. Hence, we have to invoke either changes in tumor-host relationships after a first cancer or progression to a therapy-resistant phenotype after treatment of the first primary breast cancer.

We found profound differences in the incidence trends and prognostic outlook between synchronous and metachronous bilateral breast cancer diagnosed at different ages. Adjuvant chemotherapy has a dual effect on metachronous cancer: it reduces the risk while at the same time seems to worsen the prognosis. Finally, additional research into the complex behavior of bilateral breast cancer may provide important new insights into both biologic and clinical factors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) have no conflicts statement.

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Conception and design: Mikael Hartman, Kamila Czene, Marie Reilly, Paul W. Dickman, Per Hall
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REFERENCES

1. Ries LAG EM, Kosary CL, Hankey BF, et al: (eds): SEER Cancer Statistics Review, 1975-2001. Bethesda, MD, National Cancer Institute, 2004

2. Robbins GF, Berg JW: Bilateral primary breast cancer: A prospective clinicopathological study. Cancer 17:1501-1527, 1964

3. Chen Y, Thompson W, Semenciw R, et al: Epidemiology of contralateral breast cancer. Cancer Epidemiol Biomarkers Prev 8:855-861, 1999

4. Chen Y, Semenciw R, Kliewer E, et al: Incidence of second primary breast cancer among women with a first primary in Manitoba, Canada. Breast Cancer Res Treat 67:35-40, 2001

5. Robinson E, Rennert G, Rennert HS, et al: Survival of first and second primary breast cancer. Cancer 71:172-176, 1993

6. Holmberg L, Adami HO, Ekbom A, et al: Prognosis in bilateral breast cancer: Effects of time interval between first and second primary tumours. Br J Cancer 58:191-194, 1988

7. Mattsson B, Wallgren A: Completeness of the Swedish Cancer Register: Non-notified cancer cases recorded on death certificates in 1978. Acta Radiol Oncol 23:305-313, 1984

8. Stockholms Läns Landsting: Onkologiskt Centrum. http://www.sll.se/oc

9. Larsson LG, Nystrom L, Wall S, et al: The Swedish randomised mammography screening trials: Analysis of their effect on the breast cancer related excess mortality. J Med Screen 3:129-132, 1996

10. SAS Institute: SAS/STAT User's Guide: Version 8.2. Cary, NC, SAS Institute Inc, 1999

11. Early Breast Cancer Trialists' Group: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005

12. Janschek E, Kandioler-Eckersberger D, Ludwig C, et al: Contralateral breast cancer: Molecular differentiation between metastasis and second primary cancer. Breast Cancer Res Treat 67:1-8, 2001

13. Olsson S, Andersson I, Karlberg I, et al: Implementation of service screening with mammography in Sweden: From pilot study to nationwide programme. J Med Screen 7:14-18, 2000

14. Hungness ES, Safa M, Shaughnessy EA, et al: Bilateral synchronous breast cancer: Mode of detection and comparison of histologic features between the 2 breasts. Surgery 128:702-707, 2000

15. Shen Y, Yang Y, Inoue LY, et al: Role of detection method in predicting breast cancer survival: Analysis of randomized screening trials. J Natl Cancer Inst 97:1195-1203, 2005

16. Lehman CD, Gatsonis C, Kuhl CK, et al: MRI evaluation of the contralateral breast in women with recently diagnosed breast cancer. N Engl J Med 356:1295-1303, 2007

17. Adami HO, Bergstrom R, Hansen J: Age at first primary as a determinant of the incidence of bilateral breast cancer: Cumulative and relative risks in a population-based case-control study. Cancer 55:643-647, 1985

18. Hemminki K, Vaittinen P, Easton D: Familial cancer risks to offspring from mothers with 2 primary breast cancers: Leads to cancer syndromes. Int J Cancer 88:87-91, 2000

19. Hartman M, Czene K, Reilly M, et al: Genetic implications of bilateral breast cancer: A population based cohort study. Lancet Oncol 6:377-382, 2005

20. Fisher B, Dignam J, Bryant J, et al: Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. J Natl Cancer Inst 88:1529-1542, 1996

21. Tamoxifen for early breast cancer: An overview of the randomised trials—Early Breast Cancer Trialists' Collaborative Group. Lancet 351:1451-1467, 1998

22. Polychemotherapy for early breast cancer: An overview of the randomised trials—Early Breast Cancer Trialists' Collaborative Group. Lancet 352:930-942, 1998

23. Bernstein JL, Thompson WD, Risch N, et al: Risk factors predicting the incidence of second primary breast cancer among women diagnosed with a first primary breast cancer. Am J Epidemiol 136:925-936, 1992

24. Mariani L, Coradini D, Biganzoli E, et al: Prognostic factors for metachronous contralateral breast cancer: A comparison of the linear Cox regression model and its artificial neural network extension. Breast Cancer Res Treat 44:167-178, 1997

25. Verkooijen HM, Chatelain V, Fioretta G, et al: Survival after bilateral breast cancer: Results from a population-based study. Breast Cancer Res Treat [epub ahead of print on December 21, 2006]

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